

## Production of Lactic Acid by a Local Isolate of *Lactobacillus paraplantarum*

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### Abstract:

Microbial fermentation have several advantages to produce pure lactic acid (LA) by using cheap material. Whey is a byproduct of cheese industry utilized as fermentation media with local isolate *L.paraplantarum*. LA diagnosed with high performance liquid chromatography (HPLC), Nuclear magnetic resonance (NMR) and fourier transform infrared spectroscopy (FTIR). The results of HPLC shows appearance time of peak is equal to 5.361. The acid yield is 65%. The NMR spectrum indicates that, the band (1-1.5) ppm attributed to the CH<sub>3</sub> group while the band within (4-4.5) ppm belonged to the hydroxyl protons and the proton of the second carbon atom. The FTIR spectrum of isolated LA confirmed the main peaks for lactic acid.

Key words: *L.paraplantarum*, lactic acid, Whey, fermentation.

### 1-Introduction

Lactic acid CH<sub>3</sub>CHOHCOOH (2- $\alpha$  Hydroxyl propionic acid) have one  $\alpha$ -Hydroxyl group and Asymmetrical carbon atom. LA discovered by Swedish chemist Scheele C.W.in 1780. Present in many fermented food especially in dairy products, it was the first organic acid produced commercially from microorganism due to the fermentation obtained by the presence of microorganism (Koesu et al.,2017)[1]. The physical properties of LA depend on the type of the optical isomer and in general the acid is odorless, miscible with water, alcohol and Ether, undissolved in chloroform, slightly volatile and weakly dissociated ( $K = 1.38 \times 10^{-4}$ ), most of LA salts are insoluble in water (Vaidya et al., 2005).[2]

Many new applications for LA discovered annually .In pharmaceutical applications and used in cosmetics because it is a moisturizing substance that helps to improve the skin and regenerate cells. Also used in preparation of salts, sutures, skin ointments and injection solutions inside the veins. In industrial applications, 75% are used in food processing. It is the first product employed into GRAS and FDA for food additives as emulsifiers especially in bakery products. In addition it added to improve the flavor of sweets, beverages, dairy products and also as acidification factor to the control of the pH of food and prolonged the shelf life.it is considered the basic unit for a biodegradable Polylactic acid production (Manandhar and Shah, 2020).[3]

LA product is estimated to 713 thousand tons in 2013 and expected to reach 1,906.1 thousand tons in 2020. On the other hand, The volume of investment in the global market increased in 2018 it was about 2.64 billion \$, and The annual growth rate is expected to increase to 18.7 for the period 2019-2025 according to report (Global lactic acid market size and share report, 2019-2025). (Komesu *et al.*, 2017).[1]

Commercially Lactic acid bacteria are acid-producing microorganisms, in addition to yeasts and molds, as the acid is one of the primary metabolites products, where part of the carbon used is converted into a biomass according to its source by the microorganism. And the remaining part turns into acids and other products. In general, the bacteria ferment the bentose. and hexose sugars and convert them to lactic acid, either by homogeneous fermentation or heterogeneous fermentation, and the enzymes are Phosphoketolase transaldolase, Xylose isomerase, L-and D-lctate dehydrogenase, which are the main enzymes that affect the production of lactic acid. (Juturu and Wu, 2016).[4]

LA can produce via chemical and microbial fermentation with prefer the last one. (Wee *et al.*, 2006).[5]. It is estimated that 90% of the world's annual production of LA from lactic fermentation due to additional features such as the selection of certain microbial strains to produce a pure L or D-Lactic optical isomer while industrial synthesis is a mixture of L and D-LA, less energy consumption, and the use of cheap renewable materials. (De Oliveira *et al.*, 2018)[6]

The aim of this study is produce LA by new local isolate using renewable resources cheap, pure and less pollution fermentation medium (whey).The isolated LA characterized by HPLC, MNR, and FTIR techniques.

## 2-Experemental part

### 2.1-Materials

MRS from Hi-Medium for cultivation, *L.paraplantarum YL4* new local isolate was tested and recorded to be a local Iraqi isolate from buffalo milk recorded under the name LP IQ MT622658, in Japanese Genes Bank. HCl (0.1N), Autoclave from famen company.

### 2.2- Instruments

#### 2.2.1-HPLC analysis

The technique of HPLC used in the diagnosis of LA produced from bacterial isolates. was followed in the process of separation and estimation, The device was used in the department of chemistry / ministry of science and technology / University of Baghdad. to estimate LA concentration, using a C18 column (5 microns) with dimensions  $4.6 \times 250$  mm. The injected sample size was 20  $\mu$ l and at a wavelength 360 nm. The separation was carried out using a mobile phase consisting of a mixture of acetonitrile (CH<sub>3</sub>CN) solvent with 5% phosphoric acid at room temperature and with a flow rate of 1 mL / min. Lactic acid was quantified as reported in Barbas *et al.* (2005) which calculated directly from the device.

#### 2.2.2- (H-NMR)

<sup>1</sup>H -NMR spectrum of isolated lactic acid was obtained on a Bruker spectrometer operating at 600 MHz, at room temperature. Deuterated dimethyl sulfoxide (DMSO)-d<sub>6</sub> were used as solvents, and the sample concentration was 10% (w/v). (Ma *et al.*, 2018).[8]

#### 2.2.3-(FTIR) spectrum

The Shimadzu FTIR 8400S device recorded the FTIR spectra of the LA. Spectrum was recorded at the frequency range 400-4000 cm<sup>-1</sup> using

sodium chloride cell for lactic acid. (Barrios *et al.*,2012).[9]

## 2.2-Preparation of fermentation medium

Sweet whey was prepared and used as a fermentation medium for the production of LA, milk was pasteurized at a temperature of 63 ° C for 30 minutes and left to cool to 37°C. Rinit added to milk and mixed well and waiting 30-45 minutes until curd. Then the curd was cut with a knife and left to stand for 5-10 minutes. Then the curd covered with cloth and packed into metal molds, weights were placed for the purpose of pressing to get the largest amount of whey and left for 2 hrs. Then the whey was sterilized at 113°C for 10 minutes with a steam sterilizer, then cooled to 37° C and the pH adjusted to 6.4 with (0.1N) HCl.

### 2.2.1-Inoculum preparation

First *L. paraplantarum* cultivated in MRS agar at 37 °C for 24-48 h. 50 ml of MRS-broth medium were inoculated in a 250 ml flask of pure isolate and pre-activated for 18-24 h, incubated at 37° C for 24 h.

The numbers of bacterial cells were calculated in the volume of the inoculum used by the dish-pouring method by transferring 1 ml of the activated isolate of 18-24 h grown in MRS medium to 9 ml of dilution solution and made a decimal dilution series and implanting 1 ml of each dilution into a petri dish and then pouring media was incubated at 37 ° C for 48 h (Ilmen M., 2007).[10]

### 2.2.2-Fermentation

The whey pH adjusted to 6, Inoculated with 1% inoculum with  $8 \times 10^7$  CFU / ml and incubated at 37 ° C for 3 days.

### 2.2.3-Lactic acid extraction

LA extracted according to Chakraborty and Dutta (1999). [11] ,The resulting acid stored in dark and airtight containers.

## 3. Results and Discuss

### 3.1- HPLC

LA produced from bacterial isolation was diagnosed using the HPLC device, using a standard lactic acid to prove this and shown in Figure1(a), where it was found from Figure 1(b) when compared to the similarity of the time of appearance of the produced acid with the time of emergence of the standard lactic acid. Whereas, the results of the analysis indicated that the time of appearance of the standard acid pack represented in the summit was at a retention time of 5.356 = Ret.time, as shown in Table (1), while the time of appearance of the product acid peak was at Ret = 5.361, with a percentage of 65.43%, which represents the main percentage of the produced acid. While there is another peak at the retention point = 4.759 Ret.time, with a rate of 34.56%, it was attributed to the presence of other acids. Table (2) shows the results of the analysis lactic acid sample produced from the bacteria under study.

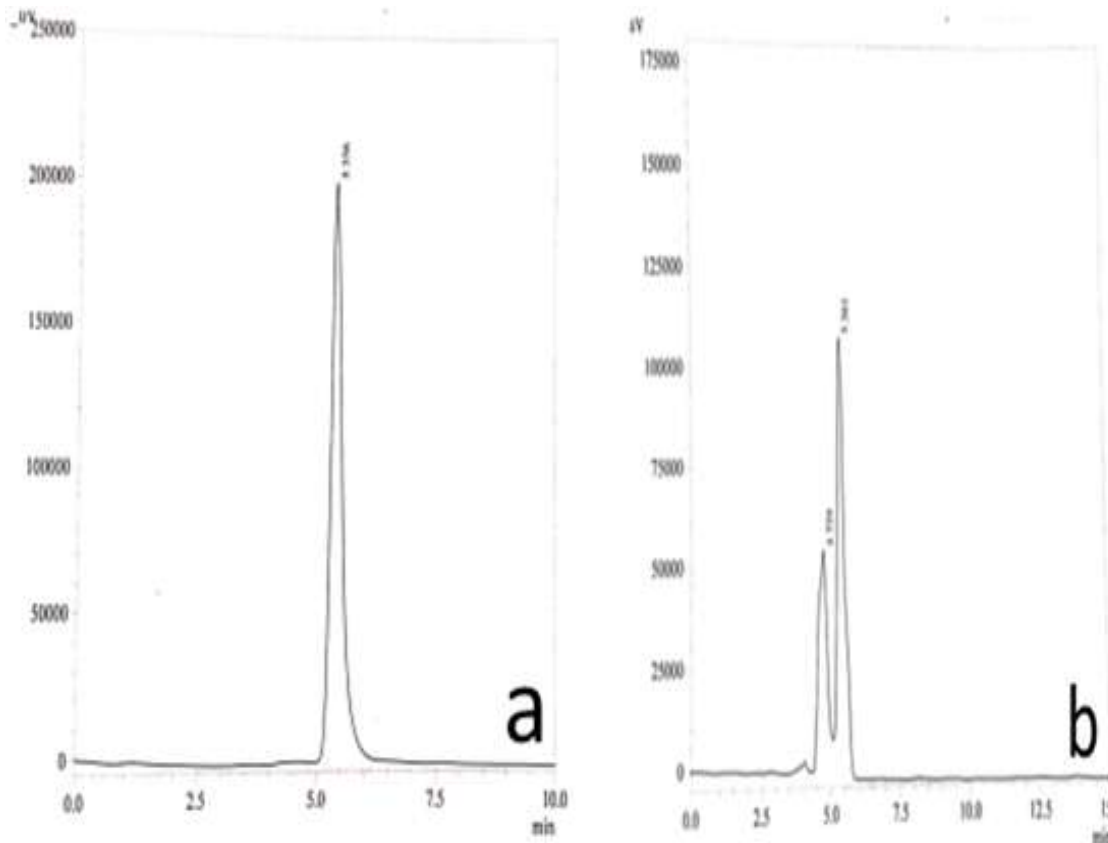


Figure1.a-HPLC for Standard (LA) b- HPLC for isolated (LA) from *L.paraplantatum*

Table1. Standard lactic acid analysis

Peak	Ret.time	Area	Heigh	Area%
1	5.356	4499	2268	100.000
Total		4499		100.000

Table2. Isolated lactic acid analysis

Peak	Ret.time	Area	Heigh	Area%
1	4.759	153962	17385	34.561
2	5.361	291517	31256	65.439
Total		445479		100.00

### 3.2- NMR analysis

It is noticed from the shape of the spectrum as a result of analyzing the lactic acid produced by the bacteria with the NMR device shown in Fig. 2 that the in situ peak (1-1.5) parts per million (ppm) belongs to the CH<sub>3</sub> group in the acid while the bundles are confined between (4 -4.5) ppm refer to the protons of the hydroxyl group and the proton of the second carbon atom, and

this is identical to what was published in (Ramanjooloo *et al.* (2009); Bouteille *et al.* (2013) [12,13].when they indicated the same locations of the methyl group (CH<sub>3</sub>) and the protons of the hydroxyl group (OH) Present in the acid produced. Note that the (D<sub>2</sub>O) solvent peak appeared at a high intensity at the site 4.8 ppm.

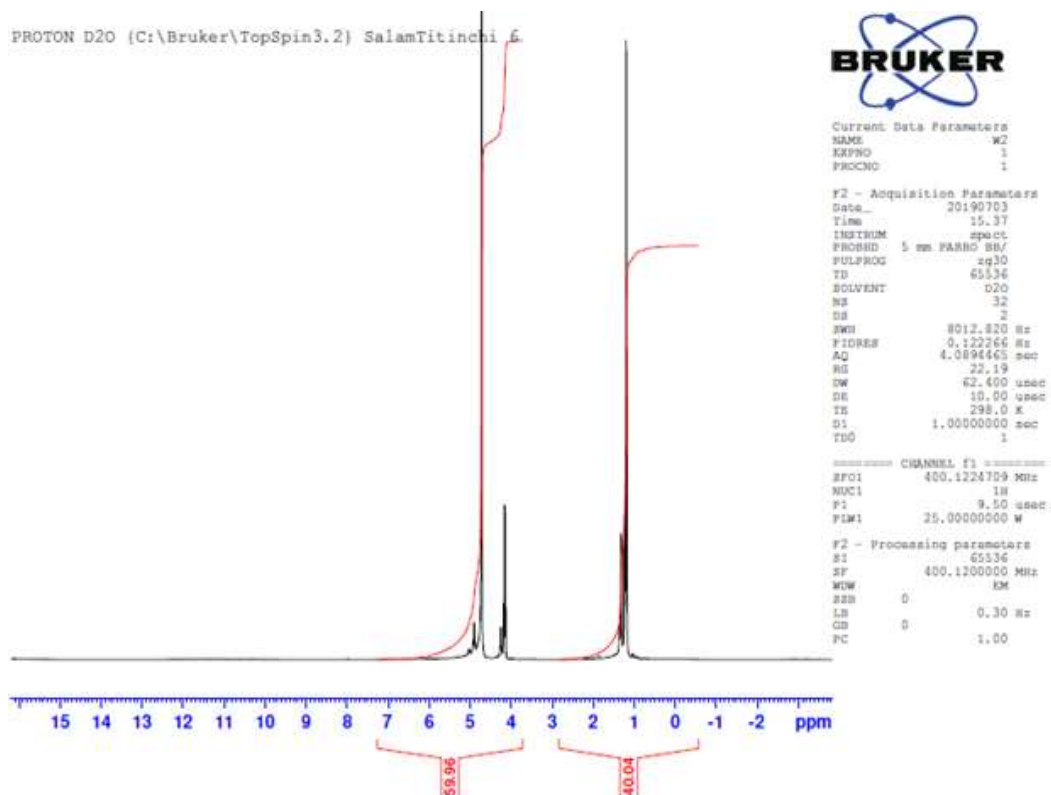


Figure 2. LA spectrum in NMR

### 3.3- FTIR analysis

FTIR analysis for LA the FTIR spectrum were studied with a range of 400-4000 cm<sup>-1</sup>. Figure 3. Shows the FTIR spectrum for the isolated acid, as the peak at 3510 cm<sup>-1</sup> indicates the frequency of the hydroxyl group (OH) in the compound, while the peaks at the site (2885, 2947, and 2993) refer to the frequency of the insistence CH. The peak at 1454 is attributed to the

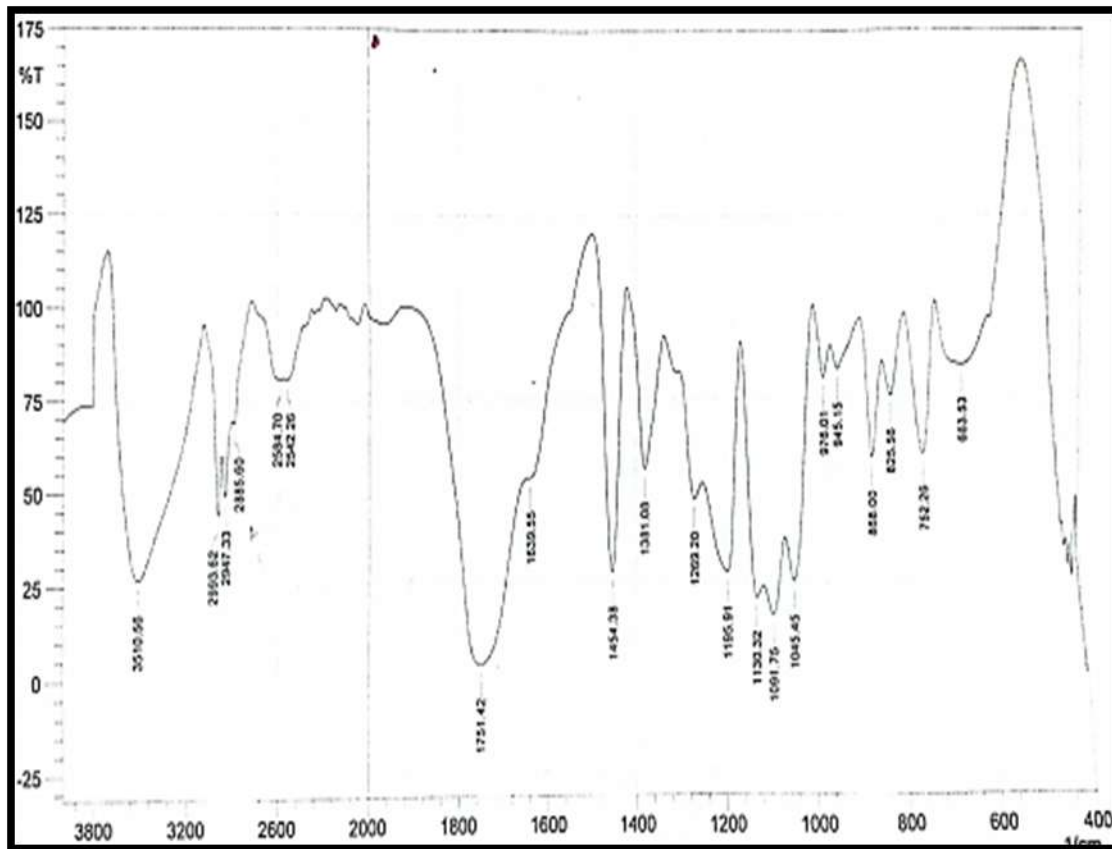
frequency of the CH<sub>3</sub> group in the composite, while the peak of the region from (1000-1300) cm<sup>-1</sup> refer to the stretching vibration frequency of the COC band, and the locations of these peaks were also indicated by the researchers (Paucean *et al.*, 2017; Bureau *et al.*, 2009).[14,15]

As for the main bundle of the compound, which is considered as a clear indication of the

formation of the acid containing the polyester bond at  $1751\text{ cm}^{-1}$ , which is attributed to the carbonyl group in the acid, and this is confirmed by Vodnar *et al.* (2010)[16] to the appearance of the carbonyl group at the same site.

On the other hand, Yoshida *et al.* (1999)[17] indicated that there are certain beams in the

FTIR spectrum that can be inferred due to the presence of the acid. Huang *et al.*, (2018) [18] indicated that an at-site peak of  $1132\text{ cm}^{-1}$  is evidence of the presence of lactic acid.



## Conclusion

The results appeared that whey media can be utilized as fermentation media to produce pure LA with high yield through short period fermentation due to contain protein and carbon source .

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